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POSTFERTILIZATION CHANGES IN THE HORSESHOE CRAB *LIMULUS POLYPHEMUS* L.

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Abstract

Postfertilization changes occurring during early development in the American horseshoe crab, *Limulus polyphemus*, have not been completely clarified in the literature. To help alleviate this problem, the present study examines the first four of twenty-one developmental stages as outlined for the Japanese horseshoe crab, *Tachypleus tridentatus*, by Sekiguchi (1973) and for *Limulus* by Brown and Clapper (1981). These four stages involve cleavage (Stages 1–3) and early blastulation (Stage 4). Cleavage consists of two parts: 1) intralecithal cleavage and 2) total cleavage. The first part (Stages 1 and 2) involves nine postfertilization events which are emphasized in this study and include the cortical reaction and three intermittant granulations. During the first two granulations, granules appear simultaneously over the entire surface of the fertilized egg. The third granulation differs from these in that granules appear locally on the embryo and gradually progress over the surface. This granulation is also characterized by a contraction wave passing over the surface. The first part of cleavage (intralecithal cleavage) ends with the appearance of nuclei near the surface (Stage 2). The second part of cleavage (total cleavage, Stage 3) then proceeds but is somewhat modified from a standard holoblastic cleavage since the planes of each division are not completed.

Introduction

In a recent review on chelicerate embryology, Anderson (1980) states that "in spite of the excellent recent work of Scholl (1977), the critical early stages of xiphosuran development are still not clear." In the present study, the sequence of dynamic postfertilization changes occurring to the *Limulus polyphemus* embryonic surface during early development has been examined. These changes have been related to cleavage, a process in *Limulus* and other xiphosurids (*Tachypleus tridentatus* and *T. gigas*) which definitely needs clarification. For this study, the authors have supported the following two-part classification of xiphosurid cleavage, as modified from a review by Anderson (1973): 1) "intralecithal cleavage" which includes all postfertilization changes occurring in Stages 1 and 2 (Sekiguchi, 1973; Brown and Clapper, 1980); and, 2) "total cleavage" which includes Stage 3 and terminates with the appearance of Stage 4 or early blastula formation. Although this present approach provides insight on the overall cleavage process with emphasis on surface changes, further investigations are necessary to link these changes with nuclear divisions and migrations.

In reference to early development of xiphosurids, earlier investigators (Brooks and Banks, 1885; Osborn, 1885; Kishinouye, 1891; Kingleys, 1892; Iwanoff, 1933) reported a series of "furrowings" which they interpreted as cleavage and which agrees

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with total cleavage as presented in this study. However, among these investigators only Kingsley (1892) described any postfertilization changes preceding total cleavage. In particular, he described a columnar layer covering the embryonic surface (granulation in the present study) that appeared and disappeared within a few hours after fertilization. He also briefly described the presence of nuclei during early development and their migration to the embryonic surface (intralecithal cleavage). Unfortunately, possibly due to the difficulty of preparing materials, he did not pursue further studies on these postfertilization events. More recently, various aspects of intralecithal cleavage have been examined (Sekuguchi, 1960; 1973; Brown and Clapper, 1980; 1981; Sekiguchi *et al.*, 1982), but the emphasis has not been on the granulation processes as outlined in the present study.

Materials and Methods

Source of animals

Adult specimens of *Limulus polyphemus* L. were shipped from the Florida Marine Biological Specimen Co., Inc., Panama City, FL, during the summer and fall months and were kept at 15°C in 150 gal Instant Ocean Aquaria containing artificial sea water (ASW) from Jungle Laboratories Corporation, Comfort, TX. The animals were maintained following the procedures previously outlined by Brown and Clapper (1981).

Gamete collection, fertilization, and early development

Gametes were collected by brief electrical stimulation (3–4 V, 0.2–1.0 mA, ac) in a region proximal to the genital pores. Semen was diluted with ASW to a 10% sperm suspension (10^9 spermatozoa/ml) and was generally used within 5 min. With wooden applicators, approximately 10–25 freshly spawned eggs were collected and placed in a plastic Petri dish containing ASW. The dish was swirled to disperse oviductal fluid, one or two drops of a sperm suspension were added, and the dish was swirled again to assure mixture of gametes. The ASW was subsequently aspirated and fresh ASW was added. To enhance the observation of surface features and nuclei, some embryos were stained (Sekiguchi, 1960) for about 2 h shortly after fertilization using one of the following vital stains: Nile blue A or neutral red (.025 mg/ml of ASW). Embryos were examined with a Wild M-5 dissecting microscope and at selected times after fertilization: 0, 15, 25, 90 min and 3, 4, 6, 12, 14.5, 20, 28, 48 h, were photographed using a Nikon camera. For all observation of develomental stages the temperature was maintained at 22–23°C.

Histology

Aliquots of fertilized eggs or embryos at the following times after fertilization: 12, 40 min and 1.5, 3, 4, 6, 9, 15, 18, 27, 48 h were removed and fixed in ice cold 2.5% glutaraldehyde buffered with 0.2 *M* sodium cacodylate (pH 7.2). Unfertilized eggs were also treated similarly. To facilitate the penetration of fixative, egg envelopes were removed from the embryos with watchmaker forceps 10–40 min after initiating the fixation procedure. After 40 min of fixation, embryos were often brittle and as a result were frequently damaged during removal of the egg envelopes. In unfertilized and recently fertilized eggs (12 min), egg envelopes adhered so tightly to the surface, they were often partially removed in order to prevent extensive damage to the surface. After removal (or partial removal) of egg envelopes, specimens were placed

in fresh fixative and stored overnight at room temperature prior to clearing with ethyl alcohol and embedding in Polysciences JB-4 plastic embedding water-soluble medium. Embryos were serial sectioned using a Sorvall M-1, stained with 1% toluidine blue with 1% borax, and subsequently examined and photographed with a Nikon camera mounted on a Zeiss WL compound microscope. The embryos were carefully monitored at each step of preparation to ensure that the above procedures were not altering the appearance of the embryonic surface.

Time-lapse cinematography

The timing of the initial appearance of postfertilization events and embryonic stages and their duration were augmented by using time-lapse cinematography. Developing embryos were photographed with an Arri-S 16 mm camera equipped with an Arri single-frame motor which exposed one frame at a time upon receiving an electrical pulse from an intervalometer. A disk intervalometer was used for filming at rates of one frame every 5 s for the first 3 h of *Limulus* development and one frame every 15 s thereafter. In addition, a clock intervalometer was used for filming at the rate of one frame per min. Shutter speed was ¼ s. The Petri dish containing embryos was placed on a sheet of black plastic, the ASW was layered with oil to prevent evaporation, and the dish was illuminated at 20 degrees with an incandescent ribbon filament microscope lamp.

Results

Stage 1, postfertilization events during intralecithal cleavage

Nine surface changes or phenomena represent this stage and are designated as "events." Although the first four events have previously been described using ultrastructural methods (Bannon and Brown, 1980; Brown and Clapper, 1980), their inclusion in this study is necessary to present a precise sequential development of *Limulus*.

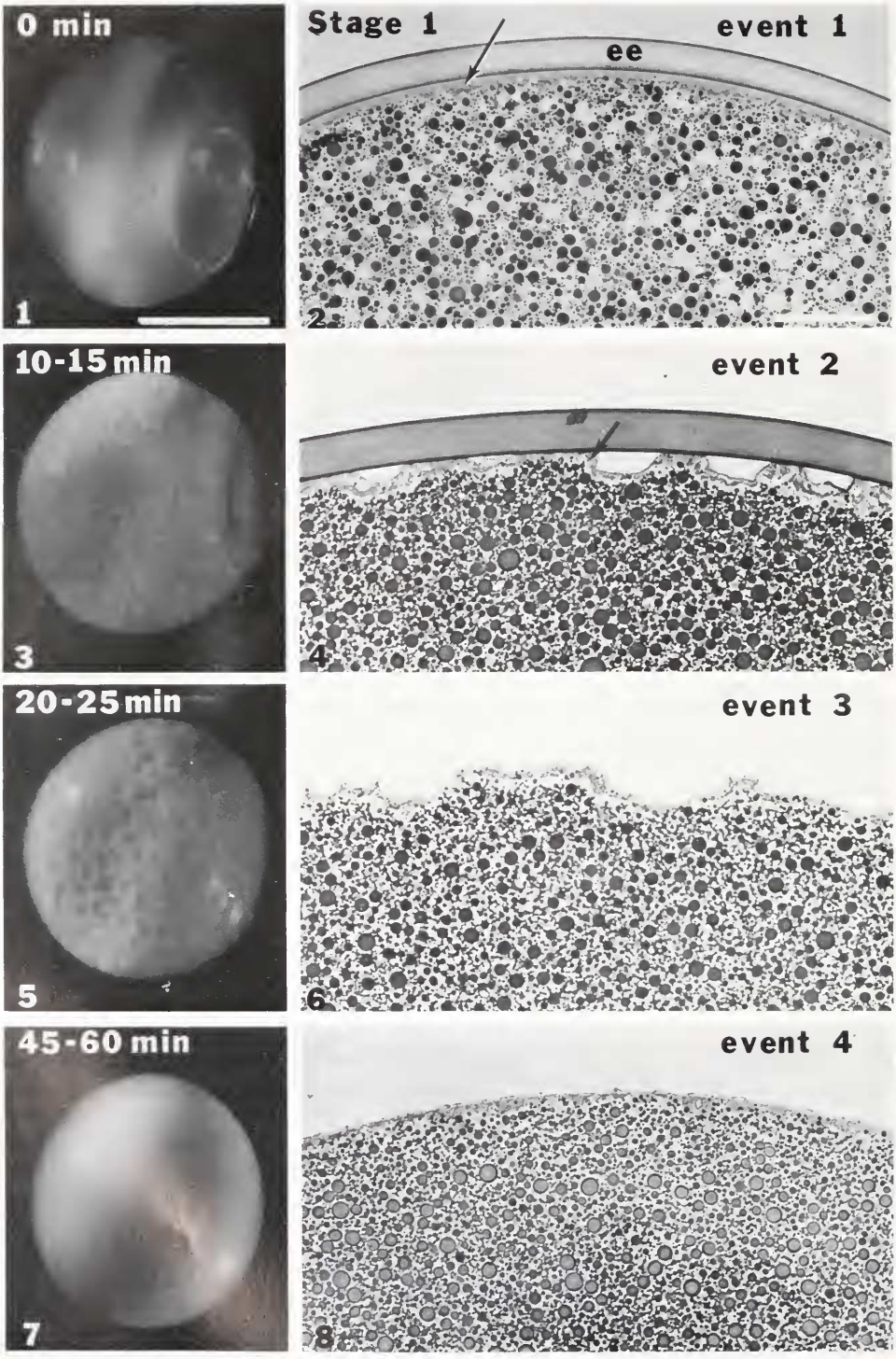
Event 1, unfertilized egg. When examining with a dissecting microscope, the egg surface (beneath the egg envelope) has a smooth appearance (Fig. 1). In histological sections, this surface is even with no bumps or depressions (Fig. 2). The thin distinct periplasm is approximately 5–10 μm thick and is similar in appearance throughout the egg periphery (Fig. 2).

Event 2, appearance of pits. Approximately 10–15 min after fertilization, small pits are distributed over the entire surface of the fertilized egg (Fig. 3) and are slowly increasing in diameter. In sections (Fig. 4), these pits are hemispherical depressions measuring approximately 80–100 μm in diameter and 30–40 μm in depth. These depressions have caused the periplasm and the underlying yolky layer to become very uneven. Besides this unevenness, the periplasm is also quite variable in thickness (5–25 μm).

Event 3, coalescence of pits. The pits continue to enlarge and coalesce with neighboring pits forming large crater-like surface areas (Fig. 5). Although the embryonic surface is still very uneven (Fig. 6), the periplasm has now become less variable in thickness (5–15 μm) when compared to event 2.

Event 4, smooth surface after the cortical reaction. By approximately 45–60 min after fertilization, the embryonic surface appears smooth and even (Figs. 7, 8). The periplasm has continued to decrease in thickness and is now once again (as in event 1) a thin peripheral layer (5–10 μm thick) around the embryo (Fig. 8).

Event 5, first granulation. The first granulation process appears about 2.5–3.0


0 min
1
Stage 1
event 1
ee
2
10-15 min
3
event 2
4
20-25 min
5
event 3
6
45-60 min
7
event 4
8

h after fertilization (Figs. 9, 10). Granules appear simultaneously over the entire embryonic surface as small nodules and increase in size eventually (10–15 min) resulting in the formation of a columnar layer (Fig. 10) which gives a corregated appearance to the periphery of the embryo (Fig. 9). Although these granules are somewhat variable in size and shape, most measure approximately 60–75 μm in length and 25–35 μm in diameter. Their contents (yolk platelets and other cytoplasmic components) are continuous with the underlying yolky material, indicating that the granules at this time are not separate membrane-bounded structures (Fig. 10). The periplasm has become greatly reduced (<5 μm thick). After being visible for approximately 1.0 h, all the granules simultaneously recede and disappear.

Event 6, smooth surface after first granulation. At 3.5–4.0 h after fertilization the embryonic surface for the second time since the cortical reaction appears smooth (Figs. 11, 12). Although similar to event 4, numerous cytoplasmic cylinders (5–10 μm wide) which are free of yolk platelets and extend approximately 30–35 μm into the cytoplasm (Fig. 12) are present and are associated with the periplasm (Fig. 12). The periplasm is much more distinct than in the previous event and forms a thin peripheral layer. This event lasts approximately 1.5 h.

Event 7, second granulation. The second granulation is initiated 5.0–5.5 h after fertilization. As in event 5, small granules appear simultaneously over the entire embryonic surface, slowly increase in size, and 10–15 min after their initial appearance form a columnar layer (Figs. 13, 14) very similar to the one in event 5 except the granules are somewhat larger and more uniform in size (approximately 75–100 μm in length and 25–40 μm in diameter). The clear cytoplasmic cylinders

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FIGURES 1–8. Stage 1, postfertilization changes during intralecithal cleavage. The approximate time after fertilization when an event is initiated is placed in the upper left hand corner of odd-numbered micrographs.

Event 1, unfertilized egg:

FIGURE 1. A typical appearance of a recently spawned egg. The large indentation on the right side is due to the packed condition of eggs in the oviduct. This indentation usually disappears in the first 30–60 min. The encompassing egg envelope is transparent and can be observed near the indentation. Beneath this envelope, the surface of the egg has a smooth appearance. Bar = 1.0 mm.

FIGURE 2. In this egg section, three distinct layers or structures are observed: the outer egg envelope (ee), the periplasm (arrow), and the inner yolky mass representing the bulk of the egg. The periplasm is distinguished by the lack of yolk platelets. Note: a peculiarity in the fixation of unfertilized eggs renders large numbers of yolk platelets transparent whereas in micrographs of fertilized eggs, these yolk platelets appear dense. Bar = 0.1 mm.

Event 2, initiation of cortical reaction and appearance of pits:

FIGURE 3. Once the egg is fertilized, the cortical reaction occurs resulting in the simultaneous appearance of numerous pits over the entire surface of the fertilized eggs. In this micrograph the pits appear as individual dark blotches.

FIGURE 4. Beneath the egg envelope in this sectioned fertilized egg, depressions (pits) have formed which have forced both the periplasm (arrow) and the surface of the yolky layer immediately below the periplasm to become uneven.

Event 3, coalescence of pits:

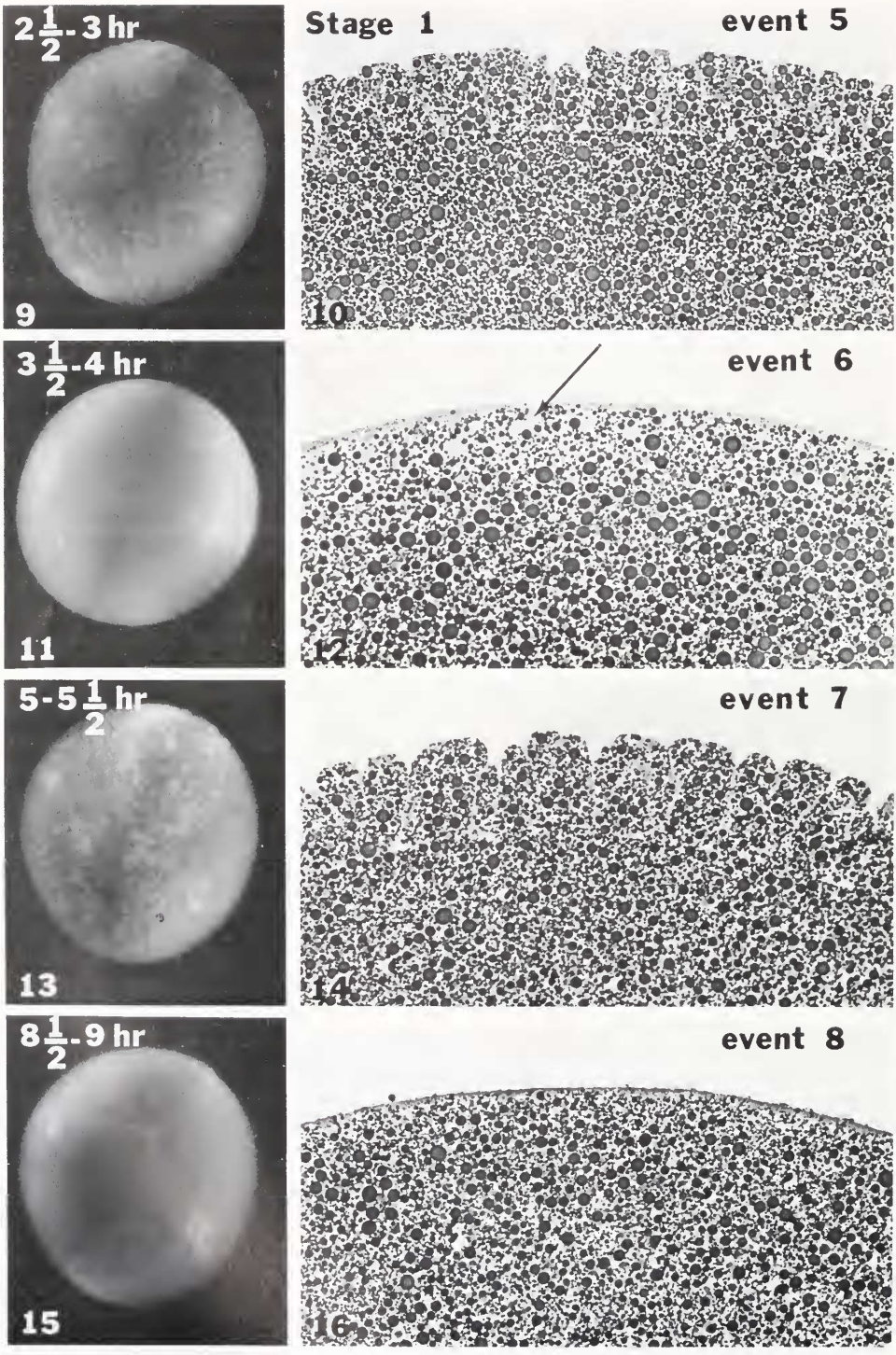
FIGURE 5. The blotchy areas representing pits have now increased in size and are variously shaped, a condition due to pits growing into one another (coalescing) and forming craters.

FIGURE 6. The surface of the fertilized egg is still uneven. In this and all following sectioned material, the egg envelope has been removed, a process which did not cause any noticeable changes in the features of the embryonic surface features.

Event 4, completion of the cortical reaction:

FIGURE 7. The fertilized egg surface is no longer distinguished by the presence of pits or craters and is characterized by a smooth appearance.

FIGURE 8. The periplasm and underlying yolky layer no longer form an uneven surface, but have an appearance similar to the sectioned unfertilized egg.


2 1/2-3 hr
9
Stage 1
event 5
10
3 1/2-4 hr
11
event 6
12
5-5 1/2 hr
13
event 7
14
8 1/2-9 hr
15
event 8
16

observed in event 6 have greatly reduced in thickness (Fig. 14). This second granulation lasts for approximately 3 h.

Event 8, smooth surface after the second granulation. At 8.5–9.0 h after fertilization, the embryonic surface for the third time since the cortical reaction has a smooth appearance (Figs. 15, 16) and very closely resembles events 4 and 6 except that the cytoplasmic cylinders (presence in event 6) are absent. The periplasm has increased to its original thickness (5–10 μm) around the embryo (Fig. 16).

Although serial sections of previous events were very carefully examined, intralecithal nuclei were observed for the first time in this event. Although not demonstrated with a micrograph, these oval-shaped nuclei (8 × 10 μm) are located within yolk platelet-free cytoplasmic masses (25–30 μm in diameter) and can be easily distinguished. In a serial sectioned embryo representing this event, 15 such nuclei were counted. Each nucleus was located approximately 300 μm (one sixth of embryonic diameter) from the surface.

Event 9, third granulation and contraction wave. Beginning 14–15 h after fertilization (Figs. 17, 18), the dynamic third granulation appears. In differing from the first two granulations, this one initiates in one locale on the embryo and slowly (approximately 1 h) spreads across the entire embryonic surface. In sections passing through the spreading edge of this granulation (Fig. 18), the individual granules are small and vary greatly in shape. As granules increase in size, a columnar layer similar to the two previous granulations forms, but is smaller in height (25–30 μm). Although the appearance of granules in this third granulation is readily observed, their disappearance has not yet been determined. Apparently, these granules persist throughout event 9 and into Stage 2 and possibly Stage 3.

When the granules blanket approximately one-third of the embryonic surface, a contraction wave becomes apparent near the origin of the granulation and precedes in the same direction as the granulation spreading edge. The contraction wave takes approximately 1 h to move over the embryonic surface and generally is prominent.

FIGURES 9–16. Stage 1: Events 5–8.

Event 5, first granulation:

FIGURE 9. Granules appear simultaneously over the entire embryonic surface giving the embryo a corrugated appearance.

FIGURE 10. Fifteen min after their initial appearance, the granules are shaped like cylinders and are continuous at their basal ends with the underlying yolky layer. As the granules form they enclose numerous yolk platelets and occupy the entire embryonic periphery.

Event 6, smooth surface after first granulation:

FIGURE 11. As the granules recede and disappear, the embryonic surface becomes smooth for the second time since the cortical reaction.

FIGURE 12. A smooth surface and irregularly-shaped cytoplasmic areas (arrow) which are extensions of the periplasm into the yolky layer are characteristics of this event.

Event 7, second granulation:

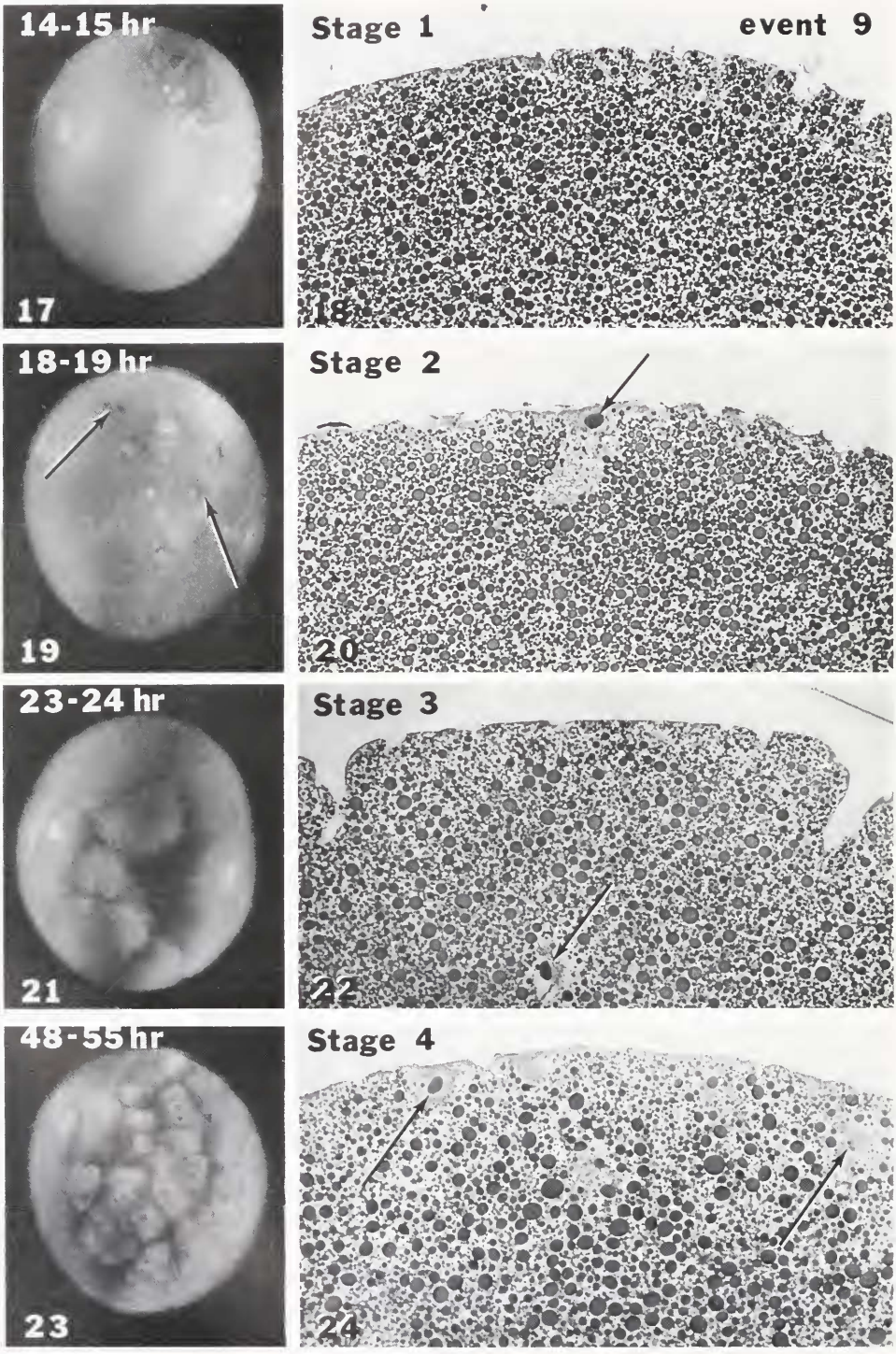
FIGURE 13. The second granulation also appears simultaneously over the surface and appears initially to be very similar morphologically to the first granulation (event 5). A major difference is the longer duration of the second granulation.

FIGURE 14. This sectioned embryo (as was the embryo for the first granulation) was fixed and prepared approximately 15 min after the initial appearance of granules. The granules for both events at this time of fixation are very similar.

Event 8, smooth surface after second granulation:

FIGURE 15. A smooth embryonic surface appears for the third time since the cortical reaction. An occasional "oil droplet" may be observed on the surface.

FIGURE 16. The thickness of the periplasm and the even surface give this sectioned embryo a resemblance to events 4 and 6, however, in the latter case, the irregularly-shaped cytoplasmic areas (Fig. 12) are absent.


14-15 hr
17
Stage 1
event 9
18
18-19 hr
19
Stage 2
20
23-24 hr
21
Stage 3
22
48-55 hr
23
Stage 4
24

The occurrence and movement of this contraction wave was overlooked in our studies and was not noticed until we viewed film produced by time-lapse cinematography.

As in event 8, intralecithal nuclei were identified. In one serial sectioned embryo, 13 such nuclei were counted. These were also located approximately 300 μm from the surface.

Stage 2, appearance of intralecithal nuclei on surface

About 18–19 h after fertilization, 12–16 intralecithal nuclei appear near the surface (Figs. 19, 20). In a sectioned embryo (Fig. 20), the cylindrical nucleo-cytoplasmic masses are in contact with the surface and extend approximately 52–62 μm into the yolky layer of the embryo. In one serial sectioned embryo, 14 intralecithal nuclei were counted. Thirteen of these were observed in close proximity to the surface and one nucleus, probably a "cleavage energid" (Anderson, 1973), was located near the center of the embryo. What may be confused with surface intralecithal nuclei are unidentifiable cell-like structures on the surface which resemble oil droplets with dark inclusions (Fig. 19).

Stage 3, "total cleavage"

Total cleavage commences when an irregular groove or furrow appears across the surface of the embryo. Upon initial examination, holoblastic cleavage appears to be occurring, however, observation of a sectioned 16-celled embryo shows that cleavage patterns are superficial (Fig. 22). Nevertheless, each blastomere of this embryo is associated with a cleavage nucleus. Also serial sections of 4 and 8 blastomere embryos (not demonstrated in this study) reveal 4 and 8 cleavage nuclei respectively associated with the blastomeres. These nuclei are believed to be daughter nuclei of the centrally located nucleus or cleavage energid described in event 9.

FIGURES 17–24. Stages 1 (Event 9), 2, 3, and 4.

Event 9, third granulation:

FIGURE 17. This granulation differs from the two previous granulations in that the origin is at one end of the embryo and slowly progresses over the entire surface. A contraction wave (not present) occurs in the same region and also passes over the embryonic surface.

FIGURE 18. The portion of the embryonic surface which demonstrates the leading edge of the granulation process.

Stage 2, appearance of intralecithal nuclei near surface:

FIGURE 19. Intralecithal nuclei (arrows) are near the embryonic surface. These nuclei are stained with Nile Blue A and appear as dark spots (arrows). As in this micrograph they are frequently paired. The upper left arrow designates one pair of nuclei while the lower right arrow demonstrates paired nuclei which are more separated than the first. The cell-like "oil droplets" observed on the surface are believed to be cytoplasmic blebs although they resemble small nucleated cells.

FIGURE 20. An intralecithal nucleus (arrow) is observed near the surface of this embryo. Surrounding the nucleus is a large amount of vesicular cytoplasm which extends into the yolky layer.

Stage 3, total cleavage:

FIGURE 21. Approximately 16 blastomeres are present in this embryo. Although total cleavage is initiated at 23–24 h after fertilization, this particular embryo has developed for 6–7 h beyond this time.

FIGURE 22. A cross section through one blastomere demonstrates that the cleavage plane is not complete. Note that the cleavage nucleus (arrow) is centralized in the blastomere block. Other nuclei which are believed to be descendants of the surface intralecithal nuclei are sometimes observed between blastomeres (not demonstrated in this micrograph).

Stage 4, early blastulation:

FIGURE 23. Nuclei in the blastomeres become obvious at the beginning of this stage. Although most blastomeres are similar in shape and size, patches of small blastomere are frequently observed.

FIGURE 24. Although the plasma membranes are difficult to observe in this section, individual blastomeres are represented by the nuclei (arrows).

Stage 4, early blastulation

Near the end of total cleavage, about 45–48 h after fertilization, when approximately 128 blastomeres are present (Fig. 23), the embryo is approaching the early blastula (Stage 4). The surface of each blastomere is so granular that internal features cannot be noticed when examining an embryo with the dissecting microscope. However, if one is patient and carefully observes this embryo, Stage 4 will appear as these granules slowly recede and the prominent nucleus (within a cytoplasmic mass) becomes obvious in each blastomere. These blastomeres vary in size, and areas of very small blastomeres can frequently be observed. In sections (Fig. 24), the blastomeres are observed only in the embryonic periphery.

Discussion

The postfertilization events during early development in *Limulus* have revealed some significant aspects previously unknown or scantily studied. The cortical reaction in fertilized eggs of horseshoe crabs was invariably overlooked by earlier investigators and only recently has been generally described (Bannon and Brown, 1980; Brown and Clapper, 1980). An interesting aspect in this study is the overall view of the cortical reaction as revealed in sectioned embryos. This reaction is a dynamic process which affects a significant portion of the embryonic peripheral layer. For example, during the reaction the periplasm and the underlying yolky layer become very uneven. As demonstrated in an earlier study (Bannon and Brown, 1980), vesicles within the periplasm fuse together and by exocytosis empty their contents into the perivitelline region causing the formation and growth of pits. However, the very large pits must involve more material in their formation than what is originally present in the periplasm. Presumably, during the cortical reaction materials are continuously passing from the yolky layer into the periplasm by means of vesicles and then are secreted to the outside. This could account for the great displacement of the yolky layer observed.

As with the cortical reaction, the three granulations (Stage 1: events 5, 7, and 9) also represent dynamic changes involving a significant portion of the embryonic periphery. The first two granulations are similar during early initiation since granules appear spontaneously and are comparable in size and shape. The second granulation lasts longer and becomes more complex (Barnum, Clapper, and Brown, in preparation). Both granulations may involve some form of plasma membrane reorganization or possible secretory process. The latter suggestion is supported since protein synthesis increases during this period (Bannon, 1981; Bannon *et al.*, 1981), and may involve the secretion of precursors for proteins of the future extra-embryonic shell.

The third granulation (Stage 1, event 9) is unique in comparison with the previous two since the first appearance of granules is localized, and the movement of a contraction wave can be demonstrated. Contraction waves during cleavage or blastulation have been described in the developing embryos of a wide variety of animals. In barnacles, for example, a set of contraction waves occur shortly after fertilization, and their disruption with chemical inhibitors causes abnormal cleavage (Lewis, 1977). In *Xenopus* (Hara *et al.*, 1980), a "surface contraction wave" on the fertilized egg precedes the first cleavage division and occurs at the same time regardless of whether egg fragments, activated unfertilized eggs, or enucleated eggs are employed, implying the presence of a biological clock mechanism in the cytoplasm near the surface. Periodic morphological changes such as contractions or wave mo-

tions in the cytoplasm of fertilized eggs have also been measured in sea urchins (Yoneda *et al.*, 1978), in ascidians (Bell, 1962) and in insects (Fullilove *et al.*, 1978; Miyamoto and van der Meer, 1982). The observation of contraction waves has stimulated considerable interest in the mechanisms of cleavage and in the control of determination in embryos of several species. Preliminary studies of this phenomenon in *Limulus* indicate that the origin of the contraction wave may be related to the determination of the embryonic axis.

The appearance of intralecithal nuclei near the surface in *Limulus* marks the end of the postfertilization events (Stage 1) and the last step in intralecithal cleavage (Stage 2). This appearance of intralecithal nuclei has also been demonstrated in embryos of *Tachypleus* by Sekiguchi (1973). Unfortunately, in both species the fate of these intralecithal nuclei is unknown, although it must be different from the nuclei involved in total cleavage since some intralecithal nuclei remain near the surface and are observed between blastomeres during total cleavage (Stage 3). Further support for this hypothesis is provided in the examination of serial sectioned embryos during late intralecithal cleavage (Stage 2) and of embryos during total cleavage (Stage 3). In one Stage 2-embryo, 13 intralecithal nuclei were observed near the surface, and one nucleus (cleavage energid) was centrally located. In serial sectioned Stage-3 embryos (not demonstrated in this paper) undergoing 4 and 8 blastomere formation respectively, 4 and 8 cleavage nuclei were observed, each midway between the center and the outer edge of the embryo and appropriately placed in each blastomere quadrant or octant. These observations suggest that the original zygotic nucleus undergoes four intralecithal nuclear divisions, and at Stage-2 all nuclei migrate to the surface except one which becomes the cleavage energid for "total cleavage." Unfortunately, the tracking of intralecithal nuclei from the zygotic stage through the cleavage process by using the traditional Feulgen reaction has been unsuccessful due to nonspecificity of this reaction on our prepared materials. Also, the surface intralecithal nuclei observed in Stage 2 appear to lose their extensive cytoplasm mass early in Stage 3, and are difficult to demonstrate thereafter. The nucleus story in the xiphosurid is far from completion and certainly needs to be clarified. We are presently pursuing this problem using more sophisticated techniques.

The cleavage phenomenon in *Limulus* embryos is very unusual since both intralecithal cleavage and total cleavage occur during early development. As this seems to be uncommon in the Animal Kingdom, a comparison of *Limulus* cleavage to other species in this study will be limited to the arachnids. Since *Limulus* has a close phyletic relationship with the arachnids, it is interesting to note that both forms of cleavages are present in this group. However, they are only found independently. For example, total cleavage is rare and limited to specialized conditions such as very small yolk-free egg (<0.5 mm) of viviparous scorpions (*cf.* Anderson, 1973). In species of arachnids having larger eggs (0.5 mm–1.9 mm), the common pattern is intralecithal cleavage ending in the formation of a blastoderm. Studies by Holm (1952) on the agelenid spider, *Agelena labyrinthica,* and by Yoshikura (1954, 1955) on the liphistiid spider, *Hepathathela kimurai,* demonstrate that after the first three nuclear divisions, the resulting eight intralecithal nuclei are distributed equally in the fertilized egg. Interestingly, this is followed by the formation of several yolk pyramids which apparently have little, if any, relationship to the nuclei. In time, the pyramids break down as the nuclei migrate to the periphery and a cuboidal blastoderm forms around the embryonic periphery. No total cleavage occurs, although as suggested by Anderson (1973), the formation of yolk pyramids is remi-

niscent of this process. Perhaps even more interesting is the formation of numerous polygonal fields in the periplasm of the otenid spider, *Cupiennius salei* (Seitz, 1966), occurring a few hours after spawning during early intralecithal cleavage. The formation of these fields certainly is similar to the granulation process in *Limulus*. In summary, intralecithal cleavage and total cleavage in *Limulus* show similarities to nuclear division, pyramid formation, and polygonal fields in arachnids. More investigation is needed, however, before a clear comparison of cleavage between the two groups can be made.

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